REMARKS

Claims 1 to 9 have been examined. Claims 1 to 9 have been amended to correct informalities. Claims 10 to 12 have been added. Support for claim 10 can be found, for example, on page 7, second complete paragraph. Claims 11 and 12 are independent form of claims 5 and 8. No new matter has been added.

Claims Rejections 35 USC §112

Claims 1 to 9 have been rejected as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regards as the invention. Claims have been amended to obviate this rejection.

Claim 1 recites:

> 1. A method for mutagenesis comprising:
> annealing one or more primers having a nucleotide sequence containing at least one mutation and a phosphorylated 5'-terminus, to a DNA template;
> elongating the annealed primer or primers by using a DNA polymerase;
> ligating the phosphorylated 5'-terminus and the elongated terminus of the primer or primers by means of a DNA ligase to synthesize a circular DNA containing said primer or primers;
> denaturing the circular DNA;
> repeating the reactions of annealing, elongating, litgating, and denaturing to amplify the circular DNA to generate DNA products including a multiple copies of a single-stranded circular DNA containing the primer or primers;
> selectively digesting the DNA products other than the single-stranded circular DNA to produce megaprimer fragments;
> annealing said megaprimer fragments to said single-stranded circular DNA; and
> elongating the annealed megaprimer fragments by using said DNA polymerase to synthesize a double stranded DNA.

Claim 1 has been amended to clarify that the reactions of annealing, elongating, ligating and denaturing to amplify the circular DNA produces a single-stranded circular DNA with the primer(s). It is also clear that inevitably there would be other residual DNA products produced at the same time, such as a single-stranded circular DNA without the primer(s), a single-stranded

circular DNA with the primer(s) annealed to the DNA template to form a double-stranded circular DNA, and a single-stranded circular DNA without the primer(s) annealed to the DNA template to form another double-stranded circular DNA (see Fig. 1 and new claim 10).

The DNA products are selectively digested such that the amplified single stranded circular DNAs are left untouched. Other DNA products such as the double stranded circular DNAs are digested to form megaprimer fragments. These megaprimer fragments are annealed (i.e. hybridized) to the single-stranded circular DNAs and elongated to form double stranded DNAs. The primer(s) is(are) left incorporated in the side of the original single-stranded circular DNAs.

Thus, claim 1 as amended is believed to have been obviated of all indefinite problems. Claims 2 and 3 have been amended also to obviate the indefiniteness. Withdrawal of this rejection is respectfully requested.

Claim Rejections – 35 USC §103

Claims 1 to 4, 6, 7, and 9 have been rejected as being unpatentable over Stemmer et al. in view of Bauer. Applicants submit that the present invention as claimed in claims 1 to 4, 6, 7, and 9 is not obvious over the cited prior art references for the following reasons.

As previously, stated, the present invention of claim 1 is a method for producing a single-stranded circular DNA with the primer(s) that have appropriate mutation(s) therein. The other residual products produced along with the single-stranded circular DNA are selectively digested to produce megaprimers. The single-stranded circular DNA is not digested by the process. The megaprimers are annealed to the single-stranded circular DNA and elongated to produce a double-stranded circular DNA with the primer(s) incorporated in the side of the original single-stranded circular DNA. In contrast, Stemmer et al.'s invention is very different from the present invention. As stated in Stemmer,

> "The method comprises (1) obtaining a first plurality of selected library members comprising a displayed polypeptide ... and an associated polynucleotide encoding said displayed polypeptide ..., and obtaining said associated polynucleotides or copies thereof wherein said associated polynucleotides comprise a region of substantially identical sequence, optionally introducing mutations into said polynucleotides or copies, and (2) pooling and fragmenting, typically randomly, said associated polynucleotides or copies to form fragments thereof under conditions suitable for PCR amplification, performing PCR amplification and optionally mutagenesis, and thereby homologously recombining said fragments to form a shuffled pool of recombined polynucleotides...." (Column 4, lines 27 to 43.)

That is, Stemmer et al. obtains associated polynucleotides of substantially identical sequences; and those polynucleotides are then fragmented and homologously recombined to from a shuffled pool of recombined polynucleotides. In the present invention of claim 1, there is no homologously recombining of polynucleotides to obtain a shuffled pool of recombined polynucleotides (see Fig. 1). The present invention of claim 1 is a method to make a multiple copies of the double stranded circular DNA with the same mutation(s) on desired positions. Megaprimers are used merely to synthesize a complementary strand of the single-stranded circular DNA (which is obtained by repeating the annealing, elongating, ligating and, denaturating reactions) to form a double-stranded circular DNA. That is, the megaprimers are annealed (i.e. hybridized) to the single-stranded circular DNA, which contains the desired mutation(s); and the megaprimers are then elongated by a polymerease to synthesize a double-stranded DNA.

Furthermore, Stemmer et al. does not disclose, teach, or suggest selectively digesting the DNA products other than the single-stranded circular DNA to produce megaprimer fragments.

Next, Applicants submit that Stemmer et al. in combination of Bauer does not render the present invention of claim 1 obvious. First, for the reasons stated above, the present invention of claim 1 is not taught or suggested by Stemmer et al. Bauer et al. discloses a circular site-directed mutagenesis but does not disclose, teach, or suggest the features of claim 1 as outlined above in rebutting Stemmer et al. Bauer et al.'s site directed mutagenesis introduces a specific mutation into a specific site by executing PCR using a pair of complementary mutant primers:

> The invention provides methods of introducing site-directed mutations into circular DNA of interest by means of mutagenic primer pairs that are selected so as to contain at least one mutation site with respect to the target DNA sequence. The mutagenic primer pairs are also selected so as to be either completely complementary or partially complementary to each other, wherein the mutation site (or sites) is located within the region of complementarity of both mutagenic primers. (Column 2, lines 39 to 47.)

The complementary pair is amplified in a single step as shown in Fig. 1(C). See also column 3, lines 38 to 42. This produces a double stranded mutagenized circular DNA (see Fig. 1(E)).

In contrast, the present invention of claim 1 introduces a mutant primer or primers to a DNA template. No complementary mutant primers are used in the present invention of claim 1. Instead, a single-stranded mutagenized circular DNA is produced in the mid-amplification process: "...repeating the reactions of annealing, elongating, litgating, and denaturing to amplify the circular DNA to generate DNA products including a multiple copies of a single-stranded circular DNA containing the primer or primers..." (claim 1). Bauer et al. at least does not teach or suggest this process. Thus, a combination of Bauer et al.'s site-directed mutagenensis with the teachings of Stemmer et al. does not render the present invention of claim 1 obvious at least for the above reasons.

For the foregoing reasons, the present invention as claimed in claim 1 is not rendered obvious by Stemmer et al. in view of Bauer et al.

Claims 2 to 4, 6, 7, and 9 depend on claim 1 directly or indirectly. Thus, at least for the same reasons as claim 1, these claims are also not obvious over the cited prior art.

Claims 5 and 8 have been rejected for depending on the rejected independent claims. Because claims 5 and 8 depend on claim 1, it is submitted that at least for the same reason as claim 1, these claims are not obvious. Furthermore, claims 5 and 8 are believed to be allowable if rewritten in independent form including all of the features of the base claim. Thus, claims 5 and 8 has been rewritten as independent claims 11 and 12.

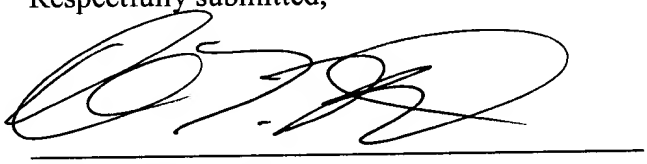
New Claims

Claim 10 is not obvious for the same reasons as claim 1. Claims 11 and 12 are allowable for being rewritten in independent form.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a check for the Petition for Extension of Time fee (three months). Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,



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Version with markings to show changes made

In the claims:.

Claims 1 to 9 have been amended as follows:

1. (Amended) A method for mutagenesis comprising [steps of]:

annealing [a DNA synthesis in which] one or more primers [which have] having a nucleotide sequence containing at least one mutation and a phosphorylated 5'-terminus, [are annealed] to a DNA template; [DNA and then subjected to]

elongating the annealed primer or primers [to an elongation reaction] by using a [thermostable high-fidelity] DNA polymerase; [,]

ligating [after which] the phosphorylated 5'-terminus and the elongated terminus of the primer or primers [are ligated] by means of a [thermostable] DNA ligase to synthesize a circular DNA containing said primer or primers;

denaturing the circular DNA;

repeating the reactions of annealing, elongating, litgating, and denaturing to amplify the circular DNA to generate DNA products including a multiple copies of a single-stranded circular DNA containing the primer or primers;

selectively digesting the DNA products other than the single-stranded circular DNA to produce megaprimer fragments; [a digestion in which said step of DNA synthesis is repeated several times to amplify the DNA containing said primers and, then, at least DNAs other than the amplified circular DNA are digested into several fragments; and]

annealing [a double-stranded DNA synthesis in which, with the several fragments obtained in said step of digestion as megaprimers, said megaprimers are annealed] said megaprimer fragments to said single-stranded circular DNA; and [synthesized in said step of DNA synthesis, followed by an elongation reaction performed]

elongating the annealed megaprimer fragments by using said [thermostable high-fidelity] DNA polymerase to synthesize a double stranded DNA.

2. (Amended) The method for mutagenesis according to Claim 1 wherein[, in said step of DNA synthesis, several of] said primers are used to introduce mutations at multiple sites simultaneously.

3. (Twice Amended) The method for mutagenesis according to Claim 1, wherein [in said step of DNA synthesis, degenerative primers are used as] said primers comprise degenerative primers to introduce random mutations at certain sites in a nucleotide sequence.

4. (Twice Amended) The method for mutagenesis according to Claim 1, further comprising:

[wherein during said step of double-stranded DNA synthesis,] before annealing the megaprimer fragments, adding an auxiliary primer complementary to a region adjacent to the nucleotide sequence in which mutations are introduced[, is added].

5. (Amended) The method for mutagenesis according to Claim 4 wherein said auxiliary primer is a T7 primer.

6. (Amended) The method for mutagenesis according to Claim 1 further comprising: [wherein, in said step of digestion,] digesting selectively the other DNA products by methylated and hemi- methylated nucleotide sequences are selectively cut.

7. (Amended) The method for mutagenesis according to Claim 1 wherein[, in said step of digestion,] DpnI is used to selectively digest the DNA products.

8. (Amended) The method for mutagenesis according to Claim 1 wherein [the] in elongating the primer or primers, a thermostable high-fidelity DNA polymerase is used, and[/or] in ligating the phosphorylated 5'-terminus and the elongated terminus, [the] a thermostable DNA ligase is used [used in said step of DNA synthesis are used in said step of double-stranded DNA synthesis].

9. (Amended) The method for mutagenesis according to Claim [1] 8, wherein[, in said step of DNA synthesis, the entire step is completed] the method is conducted in a reaction solution comprising at least said primers, said template DNA, said thermostable high-fidelity DNA polymerase and said thermostable DNA ligase.